

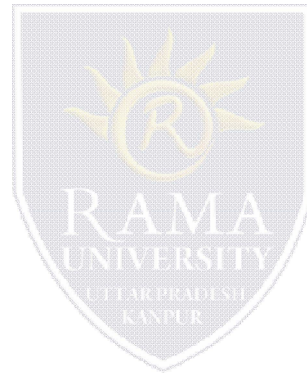


DEPARTMENT OF BIOTECHNOLOGY  
FACULTY OF ENGINEERING &  
TECHNOLOGY

# Nuclear Magnetic Resonance

## Content outline

1. Nuclear Magnetic Resonance
2. Principle of NMR
3. Instrumentation
4. Applications of NMR
5. Magnetic Resonance Imaging



## TOPIC

- In case of NMR, these entities are the nuclear magnetic spins which populate energy levels according to quantum chemical rules. Most studies in organic chemistry involve the use of  $^1\text{H}$ , but NMR spectroscopy with  $^{13}\text{C}$ ,  $^{15}\text{N}$  and  $^{31}\text{P}$  isotopes is frequently used in biochemical studies.
- The resonance condition in NMR is satisfied in an external magnetic field of several hundred mT, with absorptions occurring in the region of radio waves (frequency 40 MHz) for resonance of the  $^1\text{H}$  nucleus. The actual field scanned is small compared with the field strengths applied, and the radio frequencies absorbed are specifically stated on such spectra.

### Principle of NMR

The principle behind NMR is that many nuclei have spin and all nuclei are electrically charged. If an external magnetic field is applied, an energy transfer is possible between the base energy to a higher energy level (generally a single energy gap). The energy transfer takes place at a wavelength that corresponds to radio frequencies and when the spin returns to its base level, energy is emitted at the same frequency. The signal that matches this transfer is measured in many ways and processed in order to yield an NMR spectrum for the nucleus concerned.

## The case of the spin- $\frac{1}{2}$ nucleus

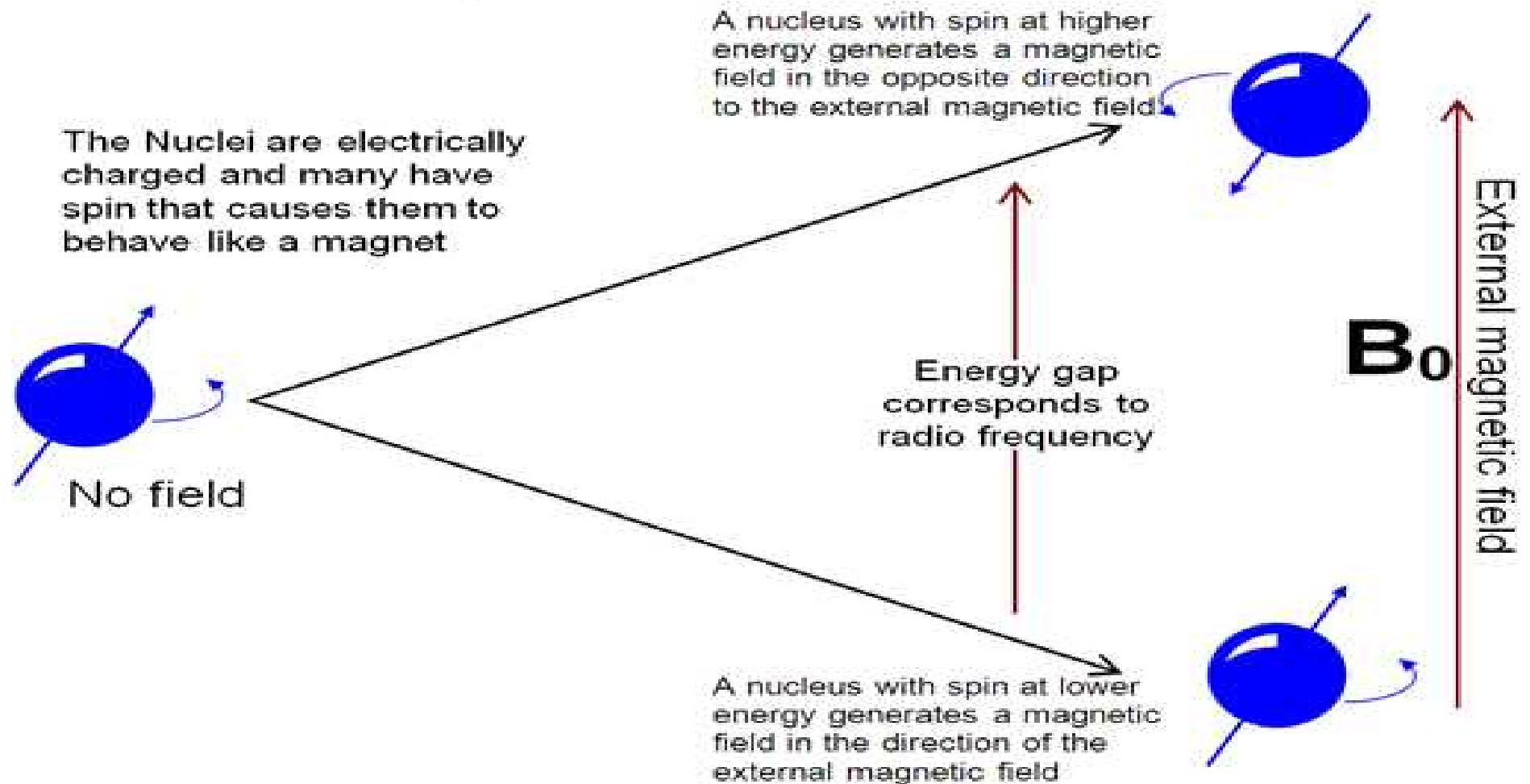
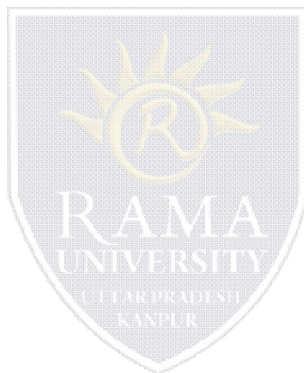
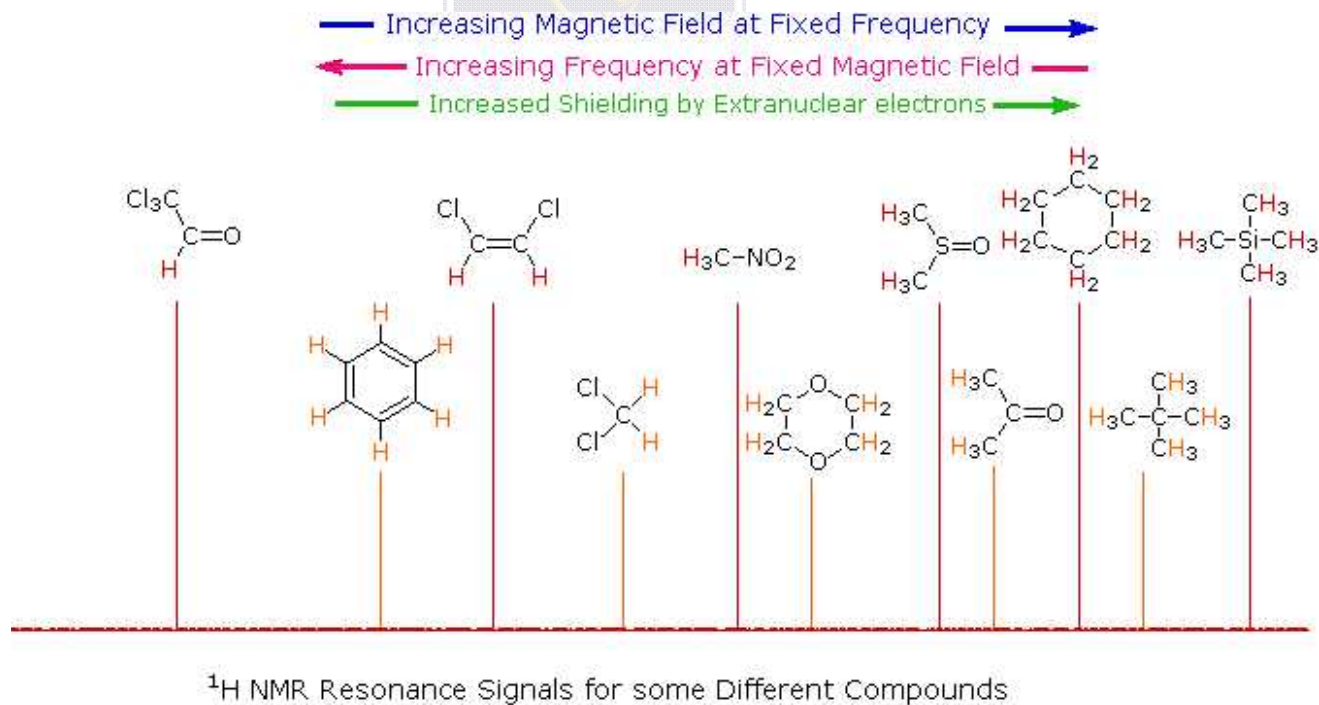


Figure in the previous slide above, relates to spin- $\frac{1}{2}$  nuclei that include the most commonly used NMR nucleus, proton ( $^1\text{H}$  or hydrogen-1) as well as many other nuclei such as  $^{13}\text{C}$ ,  $^{15}\text{N}$  and  $^{31}\text{P}$ . Many nuclei such as deuterium ( $^2\text{H}$  or hydrogen-2) have a higher spin and are therefore quadrupolar and although they yield NMR spectra their energy diagram and some of their properties are different



## Chemical shift

Unlike infrared and uv-visible spectroscopy, where absorption peaks are uniquely located by a frequency or wavelength, the location of different NMR resonance signals is dependent on both the external magnetic field strength and the rf frequency. Since no two magnets will have exactly the same field, resonance frequencies will vary accordingly and an alternative method for characterizing and specifying the location of nmr signals is needed. This problem is illustrated by the eleven different compounds shown in the following diagram. Although the eleven resonance signals are distinct and well separated, an unambiguous numerical locator cannot be directly assigned to each.



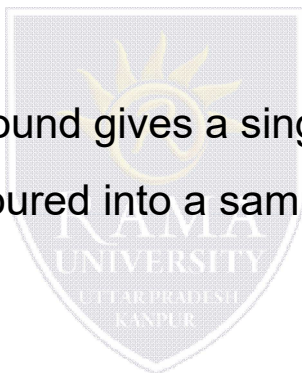
• **Tetramethylsilane**,  $(\text{CH}_3)_4\text{Si}$ , usually referred to as **TMS**, meets all these characteristics, and has become the reference compound of choice for proton and carbon nmr.

• Since the separation (or dispersion) of nmr signals is magnetic field dependent, one additional step must be taken in order to provide an unambiguous location unit. This is illustrated for the acetone, methylene chloride and benzene signals by clicking on the previous diagram. To correct these frequency differences for their field dependence, we divide them by the spectrometer frequency (100 or 500 MHz in the example), as shown in a new display by again clicking on the diagram. The resulting number would be very small, since we are dividing Hz by MHz, so it is multiplied by a million, as shown by the formula in the blue shaded box. Note that  $\nu_{\text{ref}}$  is the resonant frequency of the reference signal and  $\nu_{\text{samp}}$  is the frequency of the sample signal. This operation gives a locator number called the **Chemical Shift**, having units of parts-per-million (ppm), and designated by the symbol  $\delta$

The compounds referred to above share two common characteristics:

- The hydrogen atoms in a given molecule are all structurally equivalent, averaged for fast conformational equilibria.
- The compounds are all liquids, save for neopentane which boils at 9 °C and is a liquid in an ice bath.

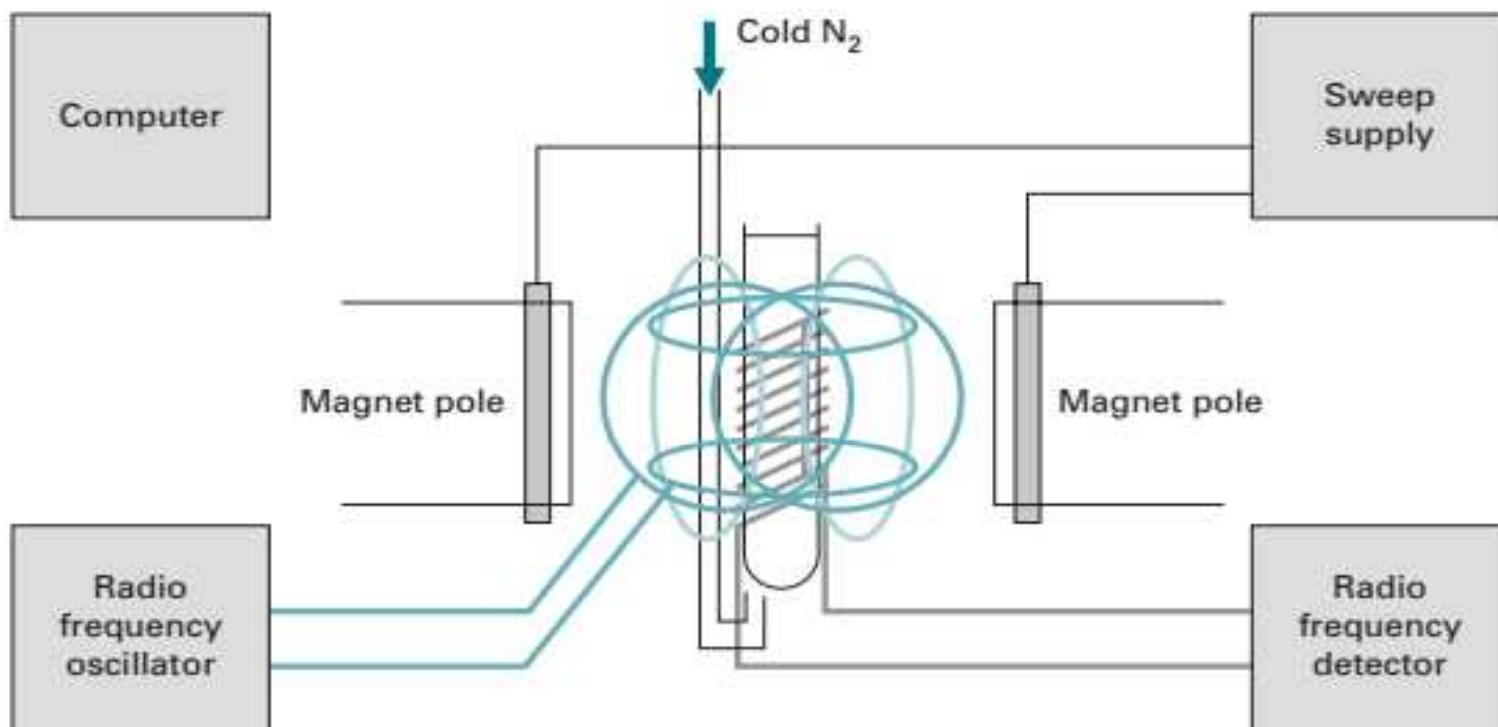
The first feature assures that each compound gives a single sharp resonance signal. The second allows the pure (neat) substance to be poured into a sample tube and examined in a nmr spectrometer





## Instrumentation

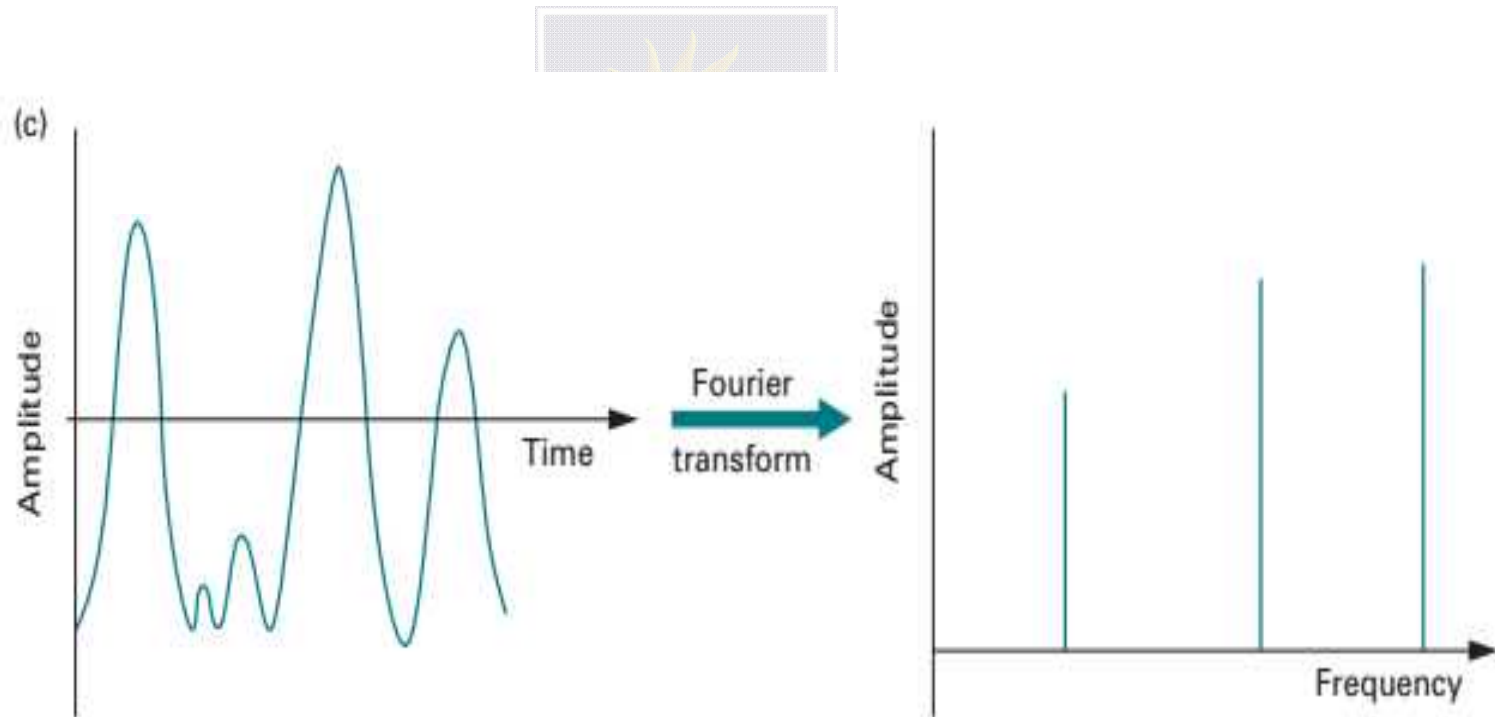
NMR uses radio frequencies in an external magnetic field to induce transition and detect signals in sample under investigation. It uses two sets of coils to generate and detect radio frequencies. Samples in solution are contained in sealed tubes which are rotated rapidly in the cavity to eliminate irregularities and imperfections in sample distribution. In this way, an average and uniform signal is reflected to the receiver to be processed and recorded. In solid samples, the number of spin–spin interactions is greatly enhanced due to intermolecular interactions that are absent in dissolved samples due to translation and rotation movements. As a result, the resonance signals broaden significantly. However, high-resolution spectra can be obtained by spinning the solid sample at an angle of  $54.7^\circ$  (magic angle spinning).



**Schematic diagram of an NMR spectrometer with cryoprobe**

## Data processing

Pulse analysis and Fourier transform methods are used for data analysis. During the scan, radiation of certain energy in the form of a sine wave is recorded. By using the mathematical procedure of Fourier transform, the 'time domain' can be resolved into a 'frequency domain'.



## Applications

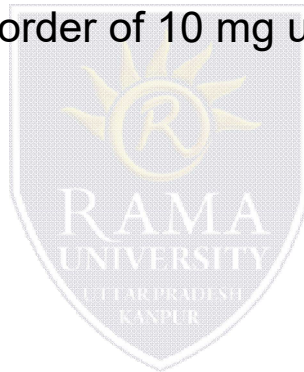
### Molecular structure determination

Traditionally, NMR spectroscopy is the main method of structure determination for organic compounds. The chemical shift provides a clue about the environment of a particular proton or carbon, and thus allows conclusions as to the nature of functional groups. Spin–spin interactions allow conclusions as to how protons are linked with the carbon skeleton. For structure determination, the fine structure usually is the most useful information because it provides a unique criterion while chemical shifts of some groups can vary over an extended range. Additionally, the signal intensity provides information as to how many protons contribute to a particular signal.

- For peptides and proteins D<sub>2</sub>O is the solvent of choice. Because the stability of the magnetic field is critical for NMR spectroscopy, the magnetic flux needs to be tuned, e.g. by locking with deuterium resonance frequencies. The use of deuterated solvents thus eliminates the need for further experiments.

## **Solution structure of proteins and peptides**

The structures of proteins up to a mass of about 50 kDa can be determined with biomolecular NMR spectroscopy. The preparation of proteins or selected domains for NMR requires recombinant expression and isotopic labelling to enrich the samples with  $^{13}\text{C}$  and  $^{15}\text{N}$ ;  $^2\text{H}$  labelling might be required as well. Sample amounts in the order of 10 mg used to be required for NMR experiments.



## **Magnetic\_resonance\_imaging**

The basic principles of NMR can be applied to imaging of live samples. Because the proton is one of the more sensitive nuclides and is present in all biological systems abundantly,  $^1\text{H}$  resonance is used almost exclusively in the clinical environment. The main distinguishing features of biological samples are its water content. It is distributed differently in different tissues, but constitutes about 55% of body mass in the average human. In soft tissues, the water distribution varies between 60% and 90%. In NMR, the resonance frequency of a particular nuclide is proportional to the strength of the applied external magnetic field. If an external magnetic field gradient is applied then a range of resonant frequencies are observed, reflecting the spatial distribution of the spinning nuclei. The number of spins in a particular defined spatial region gives rise to the spin density as an observable parameter. This measure can be combined with analysis of the principal relaxation times ( $T_1$  and  $T_2$ ).

•Tissue that contains a large amount of hydrogen, which occurs abundantly in the human body in the form of water, produces a bright image, whereas tissue that contains little or no hydrogen (e.g., bone) appears black. The brightness of an MRI image is facilitated by the use of a contrast agent such as gadodiamide, which patients ingest or are injected with prior to the procedure. Although these agents can improve the quality of images from MRI, the procedure remains relatively limited in its sensitivity.



## Test your understanding

Nuclear Magnetic Resonance uses transition of .....from ground state to excited state and back

- a. Magnetic spin and magnetic moments
- b. Electron spin and electronic moments
- c. Both (a) and (b)
- d. Neither ( a) nor (b)

Chemical shift in NMR spectroscopy is measured in

- a. Milli meter
- b. micro meter
- c. Nanometer
- d. Parts per million

Tetramethylsilane,  $(\text{CH}_3)_4\text{Si}$  is used in NMR as

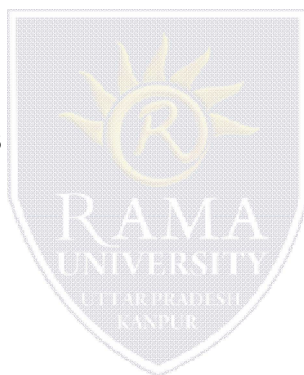
- a. Proton reference frequency
- b. Electron reference frequency
- c. Both (a) and (b)
- d. Neither (a) nor (b)

NMR is used for determination of

- a. Molecular structure of proteins and peptides
- b. Solution structure of proteins and peptides
- c. Both (a) and (b)
- d. Either (a) or (b)

Nucleids which are analyzed magnetic spin in magnetic resonance imaging ( MRI) of living samples comes from

- a. Blood
- b. Serum
- c. Water
- d. None of the above





## References & Further reading

1. Wilson, K, Walker, J., Principles and Techniques of Practical Biochemistry. 5th Ed. - Cambridge University Press,. Cambridge 1999.
2. Biotechniques, Theory & Practice: Second Edition by SVS Rana, Rustogi Publications.
3. Biochemical Methods of Analysis, Saroj Dua And Neera Garg : Narosa Publishing House, New Delhi.
4. Bioanalytical Techniques, M.L. Srivastava, Narosa Publishing House, New Delhi.

